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Steroid Receptor Coactivator 2 Is Critical for Progesterone-Dependent Uterine Function and Mammary Morphogenesis in the Mouse

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Although the essential involvement of the progesterone receptor (PR) in female reproductive tissues is firmly established, the coregulators preferentially enlisted by PR to mediate its physiological effects have yet to be fully delineated. To further dissect the roles of members of the steroid receptor coactivator (SRC)/p160 family in PR-mediated reproductive processes in vivo, state-of-the-art *cre-loxP* engineering strategies were employed to generate a mouse model ($PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$) in which SRC-2 function was abrogated only in cell lineages that express the PR. Fertility tests revealed that while ovarian activity was normal, $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mouse uterine function was severely compromised. Absence of SRC-2 in PR-positive uterine cells was shown to contribute to an early block in embryo implantation, a phenotype not shared by SRC-1 or -3 knockout mice. In addition, histological and molecular analyses revealed an inability of the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mouse uterus to undergo the necessary cellular and molecular changes that precede complete P-induced decidual progression. Moreover, removal of SRC-1 in the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mouse uterus resulted in the absence of a decidual response, confirming that uterine SRC-2 and -1 cooperate in P-initiated transcriptional programs which lead to full decidualization. In the case of the mammary gland, whole-mount and histological analysis disclosed the absence of significant ductal side branching and alveologenesis in the hormone-treated $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mammary gland, reinforcing an important role for SRC-2 in cellular proliferative changes that require PR. We conclude that SRC-2 is appropriated by PR in a subset of transcriptional cascades obligate for normal uterine and mammary morphogenesis and function.

The progesterone (P) receptor (PR) knockout (KO) mouse, in which both isoforms (PR-A and -B) were ablated, highlighted the importance of P as a pleiotropic coordinator of female reproductive biology (24). Abrogation of PR not only undermined uterine morphogenesis and function but also severely compromised the normal operation of the hypothalamo-pituitary-ovarian axis. These studies further revealed a crucial role for P signaling in mammary epithelial proliferation, an essential cellular event that enables parity-induced mammary morphogenesis to manifest in the adult. In addition, the PR KO mouse exhibited a marked reduction in mammary tumor susceptibility (25), revealing a dual role for PR-mediated epithelial proliferation in mammary tumorigenesis, as well as in normal mammary morphogenesis.

Apart from providing new cellular principles by which P influences proliferative and differentiative programs obligate for target tissue morphogenesis and tumorigenesis, two important questions have emerged from these studies regarding PR's mechanism of action for a given target tissue: (i) what are the signature molecular effectors that transduce the P signal to an appropriate physiological response, and (ii) which coregulators (coactivators and/or corepressors) are preferentially co-opted in PR-mediated transcriptional programs that induce or suppress the expression of these molecular effectors? While significant progress has been made to disclose the downstream targets of PR action in the mouse (3, 5, 8, 13, 16, 21, 37), identification of the key coregulators specifically involved in PR-mediated physiological processes is only now being realized.

Previous in vitro studies demonstrated that PR-mediated transcription is dependent on coordinate interactions with members of the steroid receptor coactivator (SRC)/p160 gene family (27). The SRC family comprises three members: SRC-1 (ERAP140, ERAP160, NcoA-1), SRC-2 (TIF-2, GRIP-1, NcoA-2), and SRC-3 (p/CIP, RAC3, AIB1, TRAM-1, ACTR) (reviewed in reference 23). Sharing strong sequence homology, all three coactivators have been shown to interact with the ligand binding domain of PR in a ligand-dependent manner. Depending on the physiological and cellular context, we posit that this interaction step serves to recruit one or more SRC members to the promoter-enhancer region of a select subset of PR target genes, the transcription of which manifests a particular physiological response to P exposure.

Determining whether one or more SRC family members occupy a coactivator role in PR-mediated physiological processes has been facilitated by generating KO mouse models for each of the coactivator members. Though the *SRC-1* KO female is viable, analysis revealed a marked reduction in the ability of its uterus to mount a decidual response (46), supporting an essential role for this coactivator in a tissue-remodeling event that is critically dependent on P signaling. The partial decidual response exhibited by the *SRC-1* KO mouse uterus suggested that additional coactivators are required to achieve the full P-induced decidual reaction. In the case of the *SRC-1* KO mouse mammary gland (46), retarded ductal

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elongation and dichotomous branching at puberty implied a role for SRC-1 in estrogen (E)-induced mammary morphogenetic effects in vivo.

Although a uterine defect was not observed in the SRC-3 KO mouse model (45), the SRC-3 KO mouse mammary gland exhibited a partial impairment in parity-associated ductal side branching and alveologenesis, a mammary phenotype that draws parallels with the PR KO mouse mammary defect (24). Moreover, the SRC-3 KO mouse mammary gland is less susceptible to mammary tumorigenesis, which reinforces the similarities between the SRC-3 KO and PR KO mammary phenotypes (19, 25) and suggests that SRC-3 may be preferentially recruited by a select subset of PR-mediated transcriptional programs that underpin P-induced mammary morphogenesis. Collectively, the use of coactivator KOs in studies of female reproductive biology suggests that while SRC-1 has evolved as an important coactivator for uterine PR action, SRC-3 is selected for a subgroup of mammary PR-mediated effects; recent reporter studies with the PR activity indicator model support this contention (12).

Unlike KOs for SRC-1 and -3, the *SRC-2* KO mouse model (referred to as the transcriptional intermediary factor 2 KO or $TIF2^{-/-}$ mouse model from here on) exhibits severe reproductive defects in both sexes (10). The $TIF2^{-/-}$ male is hypofertile, with developmental defects in spermatogenesis and age-dependent testicular degeneration. Importantly, initial analysis of the $TIF2^{-/-}$ female revealed a significant hypofertility phenotype due to partial placental hypoplasia. Subsequent studies have shown that $TIF2^{-/-}$ pups are markedly underrepresented in litters derived from $TIF2^{+/-}$ intercrosses (unpublished observations); $TIF2^{-/-}$ females resulting from such crosses are infertile.

Because of the severity of the $TIF2^{-/-}$ reproductive phenotype and the possibility that SRC-2 plays a pivotal coactivator role in PR-mediated physiological processes required for the maintenance of full reproductive capacity in the female, we generated a novel $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ bigenic mouse in which the $PR^{Cre/+}$ knock-in mouse (36) was crossed with the $SRC\text{-}2^{flox/flox}$ mouse (referred to previously as the TIF2 floxed [L2] version) (10). Because the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ bigenic model enables the postnatal ablation of SRC-2 only in cell lineages that express the PR, we were able to circumvent the embryonic, reproductive, and recently reported metabolic (15) phenotypes of the $TIF2^{-/-}$ model and evaluate the necessity of this coactivator specifically in PR-dependent transcriptional programs in the adolescent and adult. Unlike SRC-1 and SRC-3, whose coactivator properties in female reproductive biology are primarily specialized for P-initiated transcriptional programs operative in the uterus and mammary gland, respectively, we reveal SRC-2 as an indispensable PR coactivator in both target tissues.

MATERIALS AND METHODS

Generation of $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ bigenic and $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ *SRC-1* KO trigenic mice. The $PR^{Cre/+}$ knock-in mouse (36) was crossed with the $SRC\text{-}2^{flox/flox}$ (TIF2 floxed [L2 version]) mouse (10) to generate the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ bigenic mouse model. Although the *cre* gene is inserted into one PR allele, the $PR^{Cre/+}$ knock-in model is a phenocopy of the wild type (WT) (36). Similarly, the $SRC\text{-}2^{flox/flox}$ mouse, in which exon 11 (encoding the nuclear receptor interacting domain) is flanked by *loxP* sites in both copies of the *SRC-2* allele, is a phenocopy of the WT (10). In the process of generating the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ bigenic mouse, the following genotypes were also generated: $SRC\text{-}2^{flox/+}$, $SRC\text{-}2^{flox/flox}$, and the $PR^{Cre/+}$ $SRC\text{-}2^{flox/+}$ bigenic mouse, each exhibiting a WT phenotype. The $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ *SRC-1* KO trigenic mouse was created by introducing the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ bigenic mutation into the previously reported *SRC-1* KO mouse (46). Mice were housed in a temperature-controlled (22 ± 2°C) room with a 12-h light, 12-h dark photocycle and fed rodent chow meal (Purina Mills, Inc., St. Louis, MO) and fresh water ad libitum. All mice were treated humanely in accordance with institutional and IACUC guidelines for the care and handling of animals.

Hormone treatments and general mouse manipulations. Initial fertility tests entailed mating $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ females with WT virile males; WT, $SRC\text{-}2^{flox/+}$, $PR^{Cre/+}$ $SRC\text{-}2^{flox/+}$, and $SRC\text{-}2^{flox/flox}$ females were used as positive controls. The absence of litters after 6 months of mating was considered the first indication of a fertility defect.

An established gonadotropin hormone treatment regimen was used to superovulate mice (24). Briefly, 21-day-old mice were administered an intraperitoneal injection of 5 IU of pregnant mare's serum gonadotropin (VWR, West Chester, PA). Forty-eight hours later, mice received 5 IU of human chorionic gonadotropin (Pregnyl; Organon International, Roseland, NJ). Oocytes were flushed from oviducts 24 h post human chorionic gonadotropin injection.

To induce the decidualization reaction (24), ovariectomized mice were first treated with three daily subcutaneous injections of E (100 ng). After 2 days of rest, mice were treated with a daily injection of P (1 mg) plus E (6.7 ng) for 3 days. Six hours after the last E-P injection, the uterus was mechanically stimulated (with a burred needle) by lightly scratching the luminal epithelium located in the antimesometrial region. Following uterine stimulation, mice were administered a daily injection of P (1 mg) plus E (6.7 ng) for a further 5 days before uteri were isolated for weight measurement and histological examination.

For analysis of implantation sites, 6-week-old $SRC\text{-}2^{flox/flox}$ (positive controls) and $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mice were mated with WT males. At 5.5 days postcoitum (dpc), implantation sites were visualized by an intravenous injection of 1% Chicago Sky Blue 6B (Sigma-Aldrich, St. Louis, MO) dissolved in 0.9% saline, as previously described (8a).

To elicit mammary ductal side branching and alveologenesis, 9-week-old virgin mice received a subcutaneously implanted beeswax pellet (in the intrascapular region) which delivered 1 μg E and 1 mg P daily for 3 weeks (14).

Histological analysis. For immunohistochemical analysis, tissues were fixed overnight in Bouin's fixative or 4% paraformaldehyde; for immunofluorescence detection, tissues were fixed in 4% paraformaldehyde for 2 h. Immunohistochemistry analysis for PR and E receptor (ER), as well as dual-immunofluorescence analysis for PR and SRC-2, was performed as described previously (14). Briefly, the tyramide signal amplification fluorescence kit (NEL701; Perkin-Elmer Life Sciences, Boston, MA) was used for dual-immunofluorescence detection. The anti-PR antibody (A0098; a rabbit anti-human PR polyclonal antibody) was purchased from the DAKO Corporation, Carpinteria, CA; the rabbit anti-human SRC-2 antibody was obtained from Jun Qin, Baylor College of Medicine (17). Tetramethyl rhodamine isothiocyanate (red)-conjugated streptavidin and fluorescein isothiocyanate (green) were used to fluorescently detect SRC-2 and PR expression, respectively. Slides were washed and mounted in Vectashield mounting medium with 4′,6′-diamidino-2-phenylindole (DAPI; Vector Laboratories, Inc., Burlingame, CA). Images were captured with an Axioplan 2 microscope equipped for epifluorescence detection and with the appropriate tetramethyl rhodamine isothiocyanate and fluorescein isothiocyanate filters (Carl Zeiss, Jena, Germany). Captured digital images were initially processed with Metavue Software 4.6r9 (Universal Imaging, Inc., Downingtown, PA); final image montages were assembled with Photoshop CS (Adobe Systems, Inc., San Jose, CA).

To quantitate 5-bromo-2-deoxyuridine (BrdU) incorporation, mice were injected (intraperitoneally) with BrdU (Amersham Biosciences, Piscataway, NJ) at 0.1 ml/10 g of body weight at 2 h prior to sacrifice. Uteri and mammary glands were fixed, processed, embedded, and sectioned as previously described (25). For each tissue section, cell counting consisted of scoring the number of BrdU-stained cells in a random field of 1,000 cells. The average number of BrdU-stained cells in a given tissue section was obtained by taking the average obtained by counting three separate fields of 1,000 cells per section. Final counts were expressed as a percentage of epithelial cells immunopositive for BrdU. Representative sections were used in these studies, and only intensely stained nuclei were scored positive (25).

The inguinal mammary glands were processed for whole-mount staining and/or sectioning as previously described (24).

Molecular analysis. For quantitative real-time reverse transcription (RT)-PCR, total uterine RNA was isolated with Trizol reagent (Invitrogen Corporation, Carlsbad, CA). Expression levels of three marker genes upregulated in

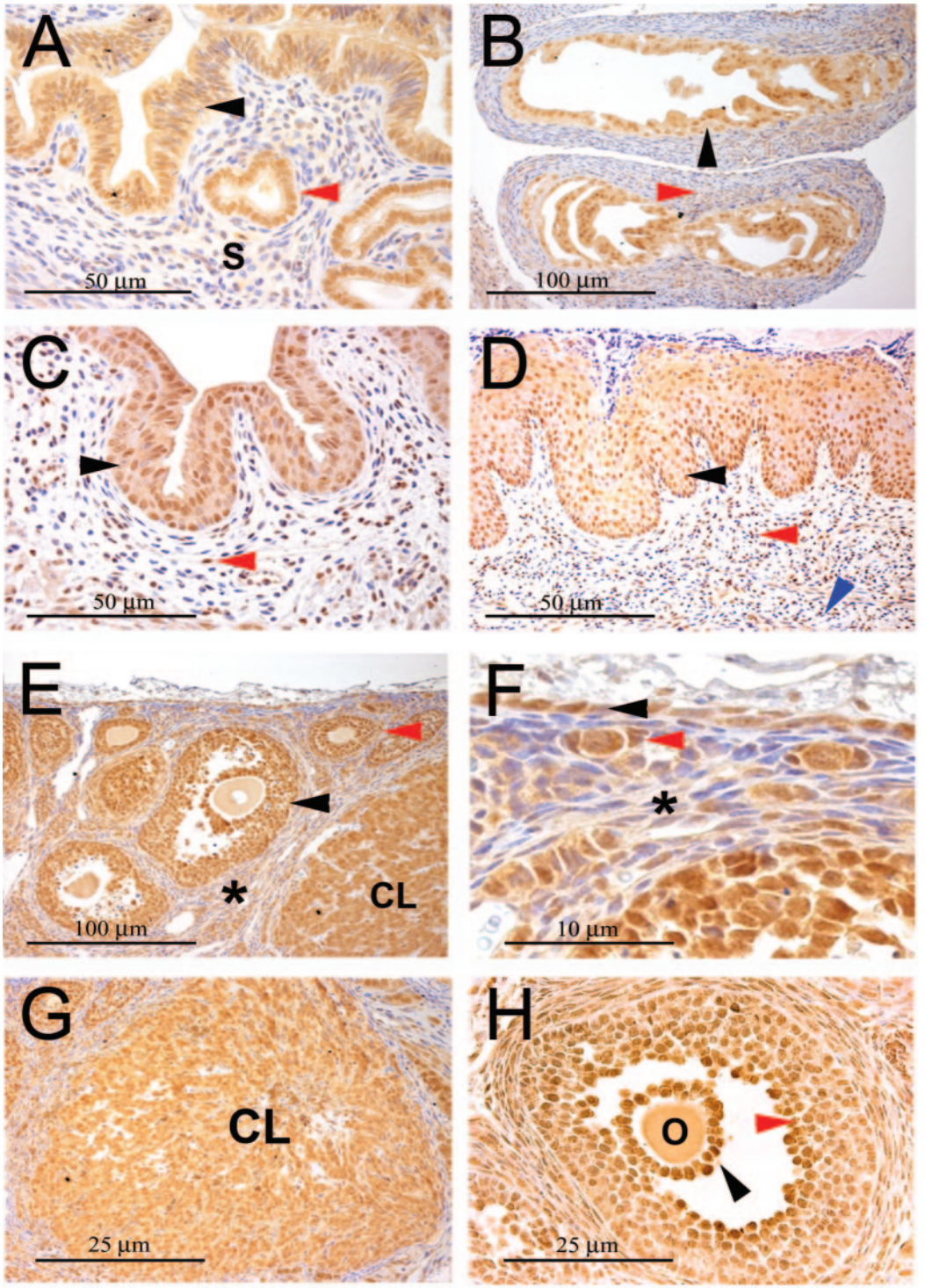


FIG. 1. SRC-2 expression in the reproductive tract of an adult WT virgin mouse. (A) Uterine SRC-2 expression is clearly observable in both luminal and glandular epithelial compartments (black and red arrowheads, respectively); lower levels of SRC-2 expression are detected in the subepithelial stroma (S). (B) Transverse section of the oviduct showing clear SRC-2 expression in tall, columnar epithelial cells (ciliated and nonciliated [or secretory]) which line the oviductal lumen (black arrowhead). Oviductal SRC-2 expression is also evident in the longitudinal and circular smooth muscle layers (red arrowhead). (C) SRC-2 expression is localized to the ectocervical epithelial and stromal compartments (black and red arrowheads, respectively). (D) Vaginal SRC-2 expression is detectable in the stratified epithelial mucosa, the vascularized submucosa, and the irregular smooth muscle layer (black, red, and blue arrowheads, respectively). (E) SRC-2 expression is present in most cellular compartments of the ovary, including the granulosa cells of the primary and secondary follicles (red and black arrowheads, respectively), the luteal cells of the corpora lutea (CL), and a subset of interstitial cells (asterisk). (F) Higher magnification revealing ovarian SRC-2 expression in the surface epithelium (black arrowhead), primordial follicle (red arrowhead), and interstitial compartment (asterisk). (G) Higher magnification of a corpus luteum revealing that all luteal cells express SRC-2. (H) In the preovulatory follicle, SRC-2 expression is detectable in the cumulus oophorus (black arrowhead), as well as in the multilaminar mural granulosa cell compartment (red arrowhead), and a low level of expression is detectable in the oocyte (O).

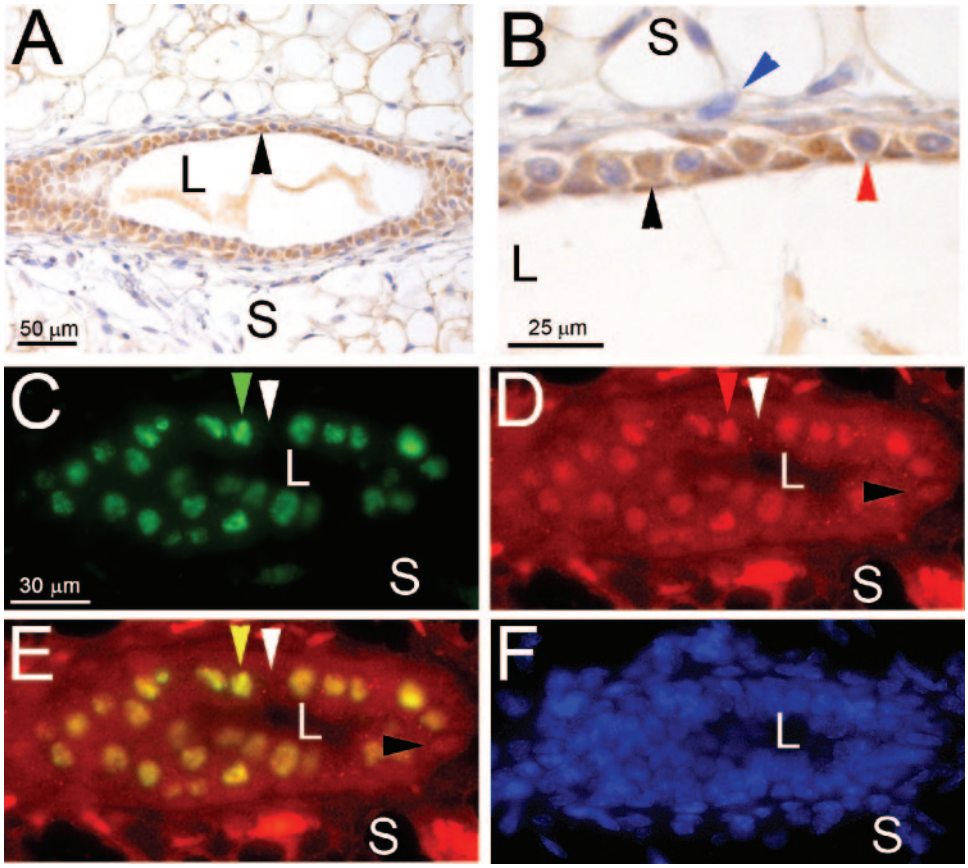


FIG. 2. Mammary SRC-2 and PR colocalize in the luminal epithelium. (A) Immunohistochemistry reveals that mammary SRC-2 expression is restricted to the luminal epithelial compartment (arrowhead) from a 12-week-old virgin mouse. L and S denote the ductal lumen and stroma, respectively. (B) Higher magnification showing that not all luminal epithelial cells express SRC-2. Black and red arrowheads highlight luminal epithelial cells scoring positive and negative for SRC-2 expression, respectively. The blue arrowhead indicates a periductal fibroblast which is SRC-2 negative. (C) Immunofluorescence reveals a subset of luminal epithelial cells that express PR (green arrowhead). The white arrowhead shows a neighboring cell scoring negative for PR expression. (D) The red arrowhead highlights the same PR-positive cell in panel C scoring positive for SRC-2; note that the cell indicated by the white arrowhead is negative for both PR and SRC-2 (compare panels C and D). The black arrowhead shows a rare luminal epithelial cell that scores positive for SRC-2 but negative for PR expression. Panel E is a merging of panels C and D (yellow, white, and black arrowheads denote mammary cells that are PR and SRC-2 positive, PR negative and SRC-2 negative, and PR negative and SRC-2 positive, respectively. (F) DAPI staining reveals all of the mammary cell types in this section.

decidualization, those for bone morphogenetic protein 2 (Bmp-2) (49), cyclooxygenase 2 (Cox-2) (22), and follistatin (18), were validated by real-time RT-PCR TaqMan analysis with the ABI Prism 7700 Sequence Detector System according to the manufacturer's instructions (PE Applied Biosystems, Foster City, CA). The TaqMan gene expression assay (catalog no. 4309169; PE Applied Biosystems) was used to perform real-time RT-PCR according to the manufacturer's instructions. Prevalidated probes and primers for murine Bmp2 (catalog no. Hs00154192_m1), Cox2 (catalog no. Mm00478374_m1), follistatin (catalog no. Mm00514982_m1), and 18S rRNA (catalog no. 4319413E; an internal control) were purchased from PE Applied Biosystems. The reaction conditions consisted of an initial activation step of 50°C for 2 min, followed by 10 min at 95°C and then 35 cycles of denaturation at 95°C for 15 s, annealing, and extension at 60°C for 1 min. All experiments were carried out in triplicate, with mRNA quantities normalized against 18S RNA with ABI rRNA control reagents.

For Western blot analysis, protein extracts were prepared from uterine and mammary tissues as outlined previously (17). Uterine or mammary gland protein (10 μg) was resolved by 4 to 15% gradient sodium dodecyl sulfate-polyacrylamide gel electrophoresis before transfer to polyvinylidene difluoride membranes (Bio-Rad Laboratories, Hercules, CA). Immunoreactive bands were detected with a polyclonal goat anti-mouse SRC-1 primary antibody (catalog no. sc-6098; 1:1,000 dilution; Santa Cruz Biotechnology, Santa Cruz, CA) and an anti-human SRC-3 monoclonal antibody (catalog no. 611105; 1:1,000 dilution; BD Biosciences, San Jose, CA). A polyclonal goat anti-human β-actin antibody was used as the loading control. For primary antibodies to SRC-1 and β-actin, the signal intensity was amplified with horseradish peroxidase-conjugated rabbit anti-goat immunoglobulin G as the secondary antibody (1:5,000 dilution; Santa Cruz Biotechnology, Santa Cruz, CA); the SRC-1 monoclonal antibody signal was amplified with horseradish peroxidase-conjugated anti-mouse immunoglobulin G as the secondary antibody. Immunoreactive bands were visualized with an enhanced chemiluminescence substrate detection kit (Pierce Biotechnology, Rockford, IL).

RESULTS

SRC-2 expression in the female reproductive tract. Although studies had reported SRC-2 expression in the human and rodent female reproductive tract (11, 28, 29, 33, 42, 47), a more comprehensive immunohistochemical study was required to determine whether P-responsive cell lineages within reproductive tissues of the cycling mouse express SRC-2. Nuclear SRC-2 immunoreactivity was detected in the uterine epithelium (luminal and glandular compartments), as well as throughout the underlying stroma and myometrium (Fig. 1A; the myometrium is not visible in this field); these cellular compartments have been shown to ex-

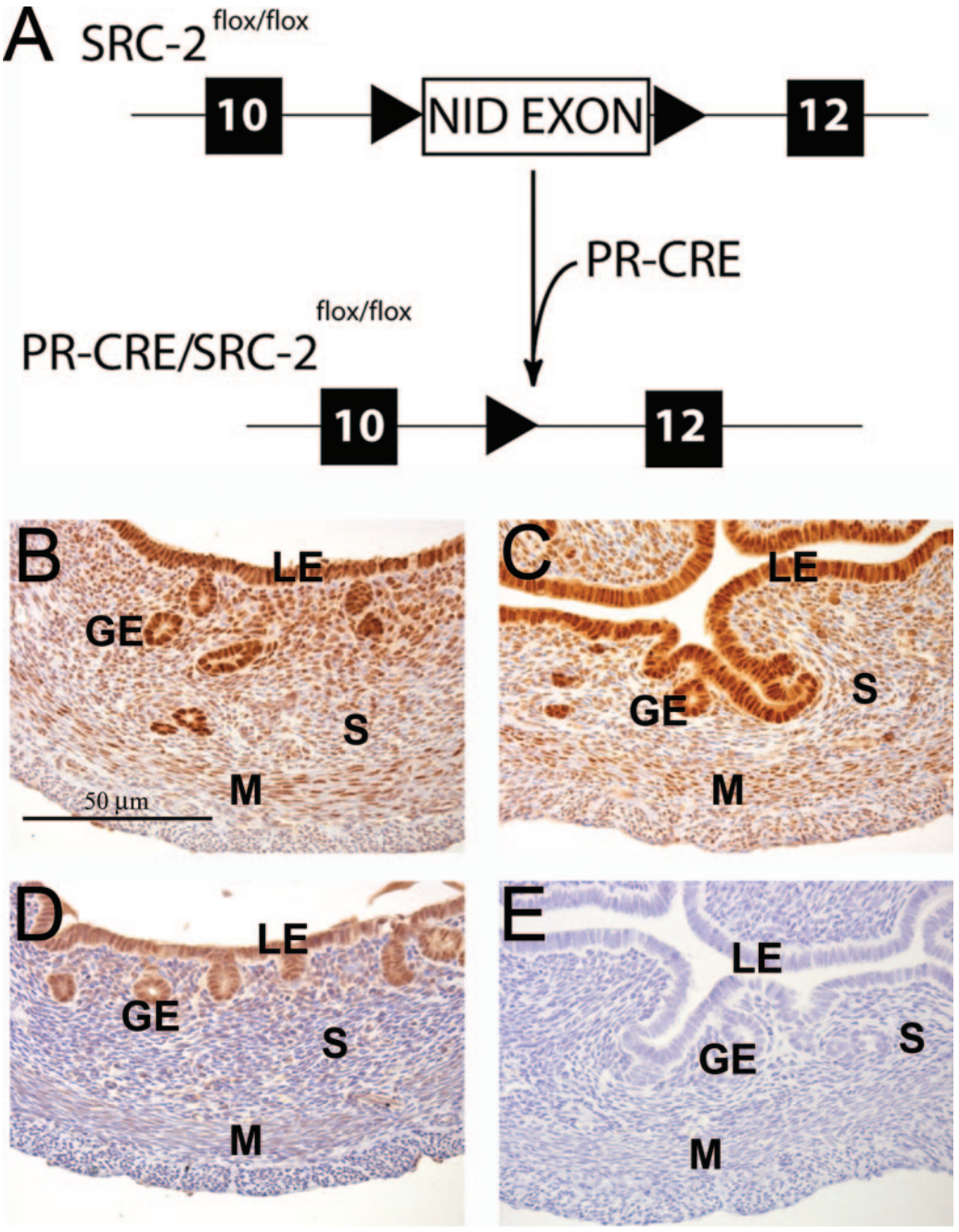


FIG. 3. Generation of the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ bigenic mouse. (A) To abrogate SRC-2 expression in PR-specific cell lineages, the $SRC\text{-}2^{flox/flox}$ mutation was introduced into the $PR^{Cre/+}$ genetic background to generate the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ bigenic mouse (see Materials and Methods). *PR* promoter-driven, Cre-mediated excision of floxed exon 11 of the *SRC-2* gene is expected to occur in all cell lineages that score positive for PR expression. (B and C) PR immunohistochemical staining of uteri obtained from ovariectomized $SRC\text{-}2^{flox/flox}$ and $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mice, respectively. (D and E) SRC-2 immunohistochemical staining of uteri derived from $SRC\text{-}2^{flox/flox}$ and $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mice, respectively. Note the absence of uterine SRC-2 expression in the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mouse uterus (E). The luminal epithelial, glandular epithelial, stromal, and myometrial compartments are indicated by LE, GE, S, and M, respectively. The scale bar in panel B applies to all of the panels.

press PR (38). In the oviductal ampullary region, significant SRC-2 expression was detected in tall, columnar epithelial cells (ciliated and nonciliated [secretory]) (Fig. 1B). Oviductal SRC-2 immunoreactivity was also observed in a subset of cells in the subepithelial smooth muscle compartment (red arrowhead). These data provide strong evidence that the spatial expression profiles of oviductal SRC-2 and PR are coincident (14). Within the ectocervix, SRC-2 expression was detected in the nonkeratinizing, stratified squamous epithelium, as well as in the stratified squamous epithelial mucosa of the vagina (Fig. 1C and D, respectively), cellular compartments that also express PR (31, 41). In both tissues,

TABLE 1. The $PR^{Cre/+}$ $SRC2^{flox/flox}$ mouse is infertile

Genotype	No. of mice tested	Mean no. of pups/litter ± SD	Mean no. of litters/mouse ± SD
WT	8	7.1 ± 0.8	4.6 ± 0.2
$SRC2^{flox/+}$	8	6.8 ± 0.6	4.4 ± 0.4
$SRC2^{flox/flox}$	8	6.9 ± 0.8	4.8 ± 0.7
$PR^{Cre/+}$ $SRC2^{flox/+}$	7	6.9 ± 0.9	4.5 ± 0.2
$PR^{Cre/+}$ $SRC2^{flox/flox}$	7	0	0

TABLE 2. The $PR^{Cre/+}$ $SRC2^{flox/flox}$ mouse ovulates normally

Genotype	No. of mice tested	Mean no. of eggs	Mean no. of fertilized eggs	% Fertilized
$SRC2^{flox/flox}$	5	13	5	38.4
$PR^{Cre/+}$ $SRC2^{flox/flox}$	8	15.75	5.5	34.9

SRC-2 immunoreactivity was localized to a cellular subgroup within the subepithelial compartment.

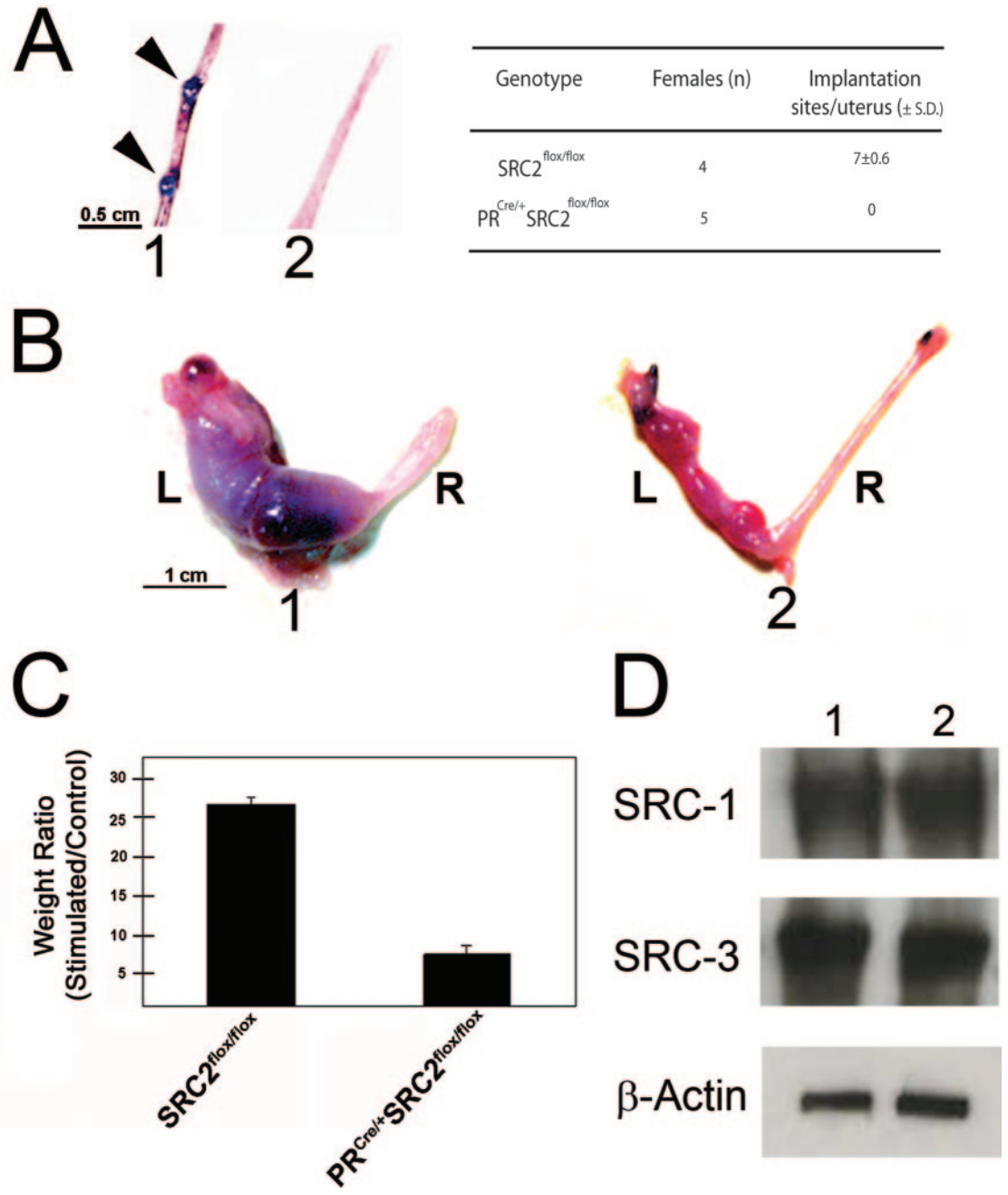
In contrast to the above, ovarian SRC-2 expression was detected in many cell types which do not express PR. For example, robust SRC-2 expression was observed in granulosa cells of both primary and secondary follicles, a subset of interstitial cells, and luteal cells of the corpora lutea (Fig. 1E). In addition, primordial follicles also express SRC-2, as does the surface epithelium (Fig. 1F), ovarian cell lineages that score negative for PR expression in the mouse (14, 34). Significant SRC-2 expression was shown for all luteal cells of the corpus luteum (Fig. 1G), an ovarian body known to be PR negative in the rodent (14, 34). In the preovulatory follicle, SRC-2 was observed not only in the cumulus oophorus but also in mural granulosa cells (Fig. 1H), with low levels of expression detected in the oocyte. The mural granulosa cell of the murine preovulatory follicle is the only ovarian cell type that expresses PR in the mouse (14, 34).

Our immunohistochemical studies demonstrate that many of the cell lineages of the uterus, oviduct, cervix, and vagina are both PR and SRC-2 positive. In the ovary, however, SRC-2 expression is observed in many cell types that are negative for PR expression, suggesting that ovarian SRC-2 may possess roles independent of PR function. Together, these expression studies provide correlative support for SRC-2's role in P-dependent responses in the uterus, oviduct, and lower reproductive tract. Although ovarian SRC-2 is expressed in a number of different cell types, the detection of SRC-2 in the mural granulosa cells of the preovulatory follicle suggests that this coactivator could facilitate intraovarian PR-mediated follicular rupture.

Mammary SRC-2 is localized to the luminal epithelial compartment. Immunohistochemistry clearly reveals that mammary SRC-2 expression is restricted to a subset of luminal epithelial cells in the gland of the adult virgin (Fig. 2A and B). Because mammary PR is also expressed in a subgroup of luminal epithelial cells (reviewed in reference 9), dual-immunofluorescence analysis was performed to determine whether mammary PR and SRC-2 colocalize to the same cell. Figure 2C and D demonstrate that mammary cells which are PR positive also express SRC-2. However, these studies also reveal that not every SRC-2-positive mammary cell scores positive for PR expression (Fig. 2D and E, black arrowheads). Mammary cells that do not express PR or SRC-2 also exist as a small subpopulation (Fig. 2D and E, white arrowheads); DAPI staining for all nuclei in the field is shown in Fig. 2F. These results suggest that SRC-2 may have a role in mammary cell types that directly respond to the P signal but that the role of this coactivator in other cell types is either to indirectly affect P action or to operate independently of this steroidal signal.

Generation of the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ bigenic mice. To identify the in vivo P-dependent reproductive and mammary responses that require SRC-2 involvement, *cre-loxP* engineering strategies were used to generate a mouse model ($PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$) in which SRC-2 function would be ablated only in cell lineages that express PR. The $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ bigenic mouse was generated by crossing of the $PR^{Cre/+}$ knock in (36) with a mouse model ($SRC\text{-}2^{flox/flox}$) in which exon 11 of the gene for SRC-2 was floxed by *loxP* sites (10) (Fig. 3A; see Materials and Methods for more details). As with the uterus from an untreated ovariectomized WT mouse, the uterus of a similarly treated $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mouse showed an identical uterine PR spatial expression profile (compare Fig. 3B and C). Despite the fact that the $PR^{Cre/+}$ knock-in mutation creates a genotype heterozygous for the intact PR allele (36), the PR immunohistochemical result indicates that PR levels are not markedly reduced from WT levels in the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ uterus (Fig. 3B and C). Though not quantitative, this result is in agreement with the observation that the $PR^{Cre/+}$ knock in is a phenocopy of the WT (36). Although SRC-2 expression was observed in all uterine cell types that express PR in the WT (Fig. 3D), as expected, SRC-2 immunoreactivity was not detected in the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ uterus (Fig. 3E). Importantly, this result demonstrated that uterine SRC-2 was ablated in PR-positive cells in accordance with the genetic design in Fig. 3A. Because the complete repertoire of uterine cell types is present in the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mouse, SRC-2 (like PR [24] and other members of the SRC family [45, 46] are not required for successful completion of the early stages of uterine development.

The $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ female is infertile. Although female and male $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ neonates were represented at the expected Mendelian frequency and exhibited normal postnatal development, the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ female was shown to be infertile (Table 1). Over a 6-month period, normal-size litters were produced at the expected frequency by WT, $SRC\text{-}2^{flox/+}$, $SRC\text{-}2^{flox/flox}$, and $PR^{Cre/+}$ $SRC\text{-}2^{flox/+}$ dams. Despite exhibiting copulatory plugs at the normal frequency, $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mice failed to produce litters during this period; in contrast, $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ males displayed normal fertility (data not shown). The observation that $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ males were fertile is interesting in that the $TIF2^{-/-}$ male reveals a severe hypofertility phenotype (10); the difference in male phenotypes between the two models underscores the tissue-selective nature of SRC-2 ablation in the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ model. The absence of a metabolic phenotype in the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mouse further highlights the phenotypic differences between the $TIF2^{-/-}$ and $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ genotypes; $TIF2^{-/-}$ mice exhibit defects in energy homeostasis (15, 32).



Genotype	Females (n)	Implantation sites/uterus (± S.D.)
$SRC2^{flox/flox}$	4	7±0.6
$PR^{Cre/+}SRC2^{flox/flox}$	5	0

FIG. 4. Impaired implantation and decidualization in the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mouse uterus. (A) Arrows indicate implantation sites in the uterus of a WT (no. 1) mouse (5.5 dpc). Implantation sites were visually scored by the localized retention of Chicago blue dye (see Materials and Methods). Implantation sites were not observed in similarly treated uteri taken from $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ (no. 2) mice at 5.5 dpc. The average number of implantation sites per genotype per the total number of mice analyzed is tabulated. (B) Gross morphological response of the left (L) uterine horn to a deciduogenic stimulus for $SRC\text{-}2^{flox/flox}$ (no. 1) and $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ (no. 2) mice. For both genotypes, the right (R) uterine horn represents the unstimulated control. (C) The average weight ratios (± the standard deviation) of stimulated (L) to control (R) horns for $SRC\text{-}2^{flox/flox}$ and $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mouse uteri are shown. (D) Uterine Western analysis of untreated adult virgin $SRC\text{-}2^{flox/flox}$ (lane 1) and $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ (lane 2) mice reveals no difference in the expression levels of uterine SRC-1 and SRC-3 between the two genotypes (the loading control was β-actin).

Because (i) SRC-2 is expressed in the ovary (Fig. 1E to H) and (ii) the PR KO exhibits an ovulation defect (24), our first line of study was to determine whether the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ ovary can undergo ovulation in response to exogenous gonadotropins. The $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mouse ovary was shown to ovulate normally (Table 2). The average yield of oocytes collected in the oviduct of the superovulated $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mouse was comparable to that observed in the similarly treated $SRC\text{-}2^{flox/flox}$ sibling, indicating that SRC-2 (like SRC-1 and -3) is not required for PR-mediated follicular

rupture. Moreover, the percentage of $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ oocytes fertilized by WT males was equivalent to that observed for $SRC\text{-}2^{flox/flox}$ oocytes (Table 2). Furthermore, the fact that oocytes and embryos were observed in the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ oviduct suggests that oviductal SRC-2 (Fig. 1B) is not required for oocyte oviductal transport.

A severe uterine defect in the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mouse. The absence of implantation sites in the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ uterus following 5.5 dpc demonstrated not only that a uterine defect accounts for the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ infertility phenotype but that this defect blocks the early progression of multistage uterine cellular processes that establish the maternofetal interface (Fig. 4A). Triggered by embryo apposition, attachment, and subsequent trophoblast invasion, the uterus undergoes a decidual reaction that is completely dependent on P signaling (24). In the absence of embryo implantation, however, an artificial decidual response can be induced in an appropriately E-P-treated uterus through the use of a deciduogenic stimulus (i.e., a burred needle) (24; see Materials and Methods). In the case of the steroid-treated uterus of the $SRC\text{-}2^{flox/flox}$ mouse, a full decidual response was clearly observed in the left stimulated horn (Fig. 4B and C). By contrast, the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ uterus displayed a partial decidual response to the deciduogenic stimulus (Fig. 4B and C), suggesting not only that PR-mediated transcription requires SRC-2 to launch a full uterine decidual response but that other coactivators are required in concert with SRC-2 in P signaling pathways to ensure complete decidualization. Because the *SRC-1* KO uterus also exhibits a partial decidual response (46) and because SRCs (through increased expression) have been shown to compensate for the absence of another (46), Western analysis was performed to rule out the possibility that the partial decidual response exhibited by the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ uterus may be indirectly linked to a parallel reduction in uterine SRC-1. Figure 4D clearly shows that uterine SRC-1 levels are not altered in the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ model, supporting the conclusion that the partial decidual response phenotype is directly attributable to loss of SRC-2.

Compared to the $SRC\text{-}2^{flox/flox}$ positive control, real-time PCR revealed a significant reduction in the expression levels of a number of decidualization markers (18, 22, 49) in the partially decidualized $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ uterine horn (Fig. 5). The negligible induction of Bmp2 compared with the partial induction of Cox2 and follistatin suggests that uterine SRC-2 is essential for the induction of pathways that lead to Bmp 2 expression but that additional coregulators may be responsible for elaborating the Cox2 and follistatin expression pathways, which collectively are required for the full decidual reaction.

The facts that the partial decidual response exhibited by the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ bigenic mouse mirrors the partial decidual response displayed by the *SRC-1* KO mouse uterus (46) and a subset of decidual molecular markers is partially induced in the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mouse uterus suggested that, from the full spectrum of coactivators in the uterine cell, SRC-2 and SRC-1 may have been uniquely coselected to enable full P-dependent decidualization to occur. To address this proposal, the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mutation was introduced into the *SRC-1* KO genetic background to generate the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ *SRC-1* KO trigenic model. Figure 6A and B show that while the $SRC\text{-}2^{flox/flox}$ mouse can manifest a full decidual response, the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ *SRC-1* KO trigenic mouse failed to show even a partial decidual response. The absence of a decidual response in the trigenic uterus provides strong in vivo support for the proposal that SRC-1 and SRC-2 are necessary and sufficient to ensure a complete P-induced decidual reaction.

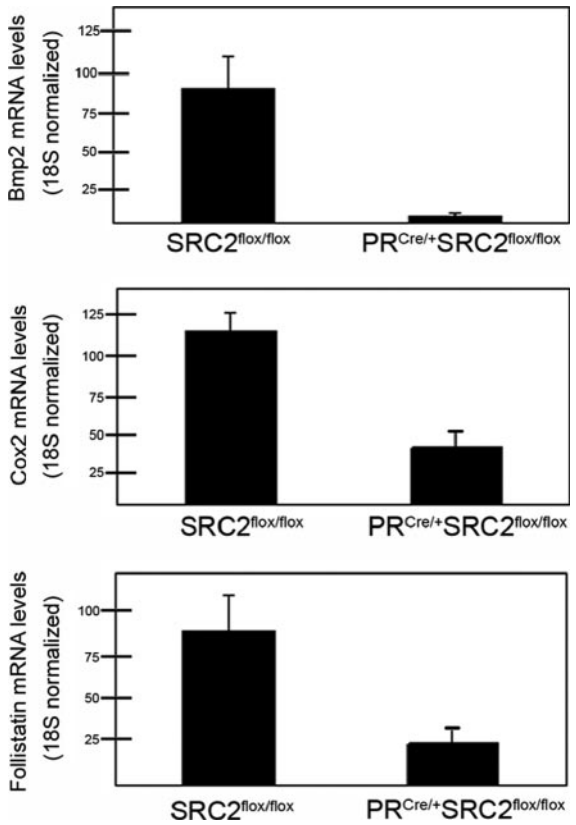


FIG. 5. Real-time PCR reveals significant decreases in expression levels for decidualization molecular markers in the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mouse uterus. Bone morphogenetic protein 2 (Bmp2) transcription was significantly reduced in the partially decidualized $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mouse uterine horn, whereas Cox2 and follistatin were partially reduced.

SRC-2 is not required for E-induced uterine proliferation. Since ER is expressed in the same uterine cell types that express PR (compare Fig. 7A and B with Fig. 3B and C) and that SRC-2 has been implicated in ER-mediated signaling (7, 44), the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ uterus was tested to determine whether loss of SRC-2 in this cell type compromises established ER-mediated signaling events that lead to uterine luminal epithelial proliferation. Compared to uteri from ovariectomized hormonally untreated mice (Fig. 7C and D), uteri from E-treated ovariectomized WT and $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mice show an equivalent proliferative response to E (as measured by BrdU incorporation; compare Fig. 7E and F). Furthermore, the increase in E-induced epithelial proliferation in the uteri of mice with both genotypes was accompanied by the classic uterotropic response, consisting of a disorganization (or tufting) of the luminal epithelial compartment with columnar epithelial cells displaying increased hypertrophy and hyperplasia accompanied by an underlying edematous stroma, physiologi-

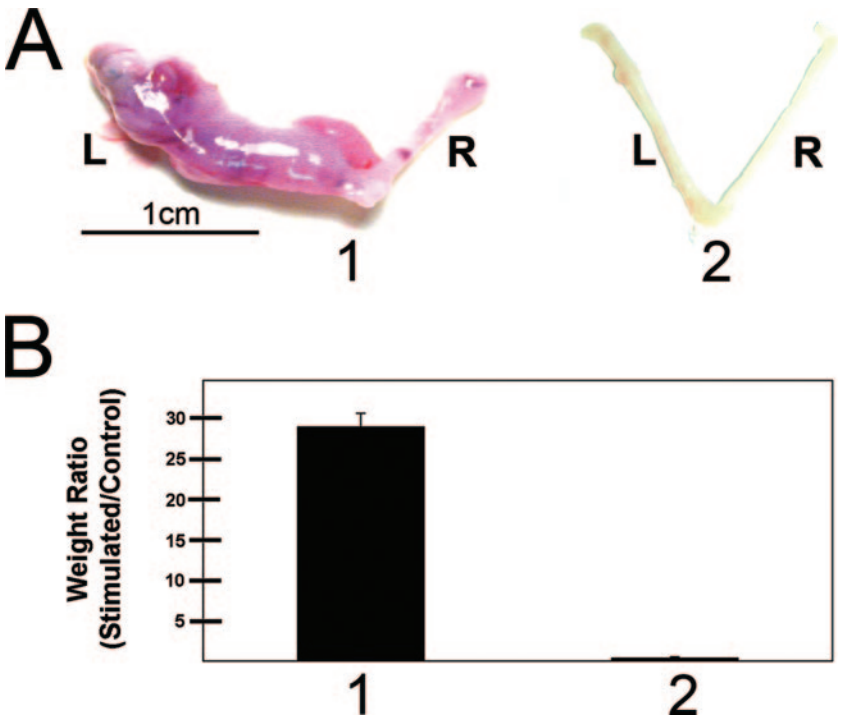


FIG. 6. Absence of a decidual response in the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ *SRC-1* KO trigenic mouse. (A) The stimulated left (L) uterine horn of the $SRC\text{-}2^{flox/flox}$ mouse (no. 1) shows full decidualization (the right [R] horn is the unstimulated horn). By contrast, the similarly treated $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ *SRC-1* KO trigenic mouse uterus (no. 2) fails to mount a decidual response in the left (L) uterine horn. (B) Graph of the normalized weight ratios (± standard deviation) of stimulated (L) to control (R) horns for the $SRC\text{-}2^{flox/flox}$ mouse (no. 1) and the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ *SRC-1* KO trigenic mouse (no. 2).

cal hallmarks of unopposed E action. These data support the conclusion that SRC-2 has evolved to serve P (rather than E)-initiated transcriptional programs in the uterus. In keeping with its coactivator activity in the uterus, SRC-2 was shown not to be required for P suppression of E-induced uterine epithelial proliferation or for P downregulation of uterine PR expression (data not shown).

Branching morphogenesis is severely impaired in the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mammary gland. Like the PR KO mouse mammary gland (24), the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mouse mammary gland undergoes normal development to adulthood (data not shown). This observation suggests that, like PR, mammary tissue-derived SRC-2 is not required for early postnatal development of the mammary gland. Since SRC-2 is expressed in PR-positive mammary cells (Fig. 2), we reasoned that this coactivator may have a role in PR-mediated signaling that leads to ductal side branching and alveologenesis in the adult gland. To test this hypothesis, the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mouse was treated with a standard 3-week E-P treatment regimen, which induces ductal side branching and alveologenesis in the WT gland. Unlike the hormone-treated WT gland, the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ gland failed to elicit full ductal side branching and alveologenesis in response to E-P treatment (Fig. 8A to F). As reported for the PR KO phenotype, the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ epithelium did not undergo proliferation even in the presence of mammary PR (Fig. 8G to I). Despite normal levels of mammary SRC-1 and -3 in the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mouse (Fig. 8I), these data highlight an indispensable role for SRC-2 function in P-induced mammary ductal side branching and alveologenesis, cellular processes that normally occur with pregnancy onset.

DISCUSSION

The observations that all members of the SRC family can directly interact with PR and that distinct SRC combinatorial assemblies can activate specific gene sets (27) provide strong support for the assertion that differential recruitment of SRC family members represents an important mechanism by which the PR differentially mediates its effects in vivo. Beyond identifying overall tissue preferences for individual SRCs, KO studies have begun to assign SRC members to select progestin responses in vivo. Though fertile and viable, the *SRC-1* KO mouse displays a partial decidual response in the uterus (46), suggesting that this coactivator (with others) is required for full manifestation of this morphogenetic response which is contingent on an intact P signal. SRC-3 KO females are fertile and viable but exhibit partial impairment of hormone-induced mammary ductal side branching and alveologenesis (45), epithelial changes that are absent in the PR KO gland (24). Together, these studies support the contention that SRC-1 and -3 are co-opted for a subset of PR-mediated transcriptional changes in the uterus and mammary gland, respectively. Unlike SRC-1 and -3, recent studies of the $TIF2^{-/-}$ mouse disclosed severe impairments of fertility in both sexes (10). In this study, we have evaluated the possible autonomous, interacting, or redundant coactivator roles of SRC-2 in female reproductive processes that depend on P signaling. To achieve this goal, a

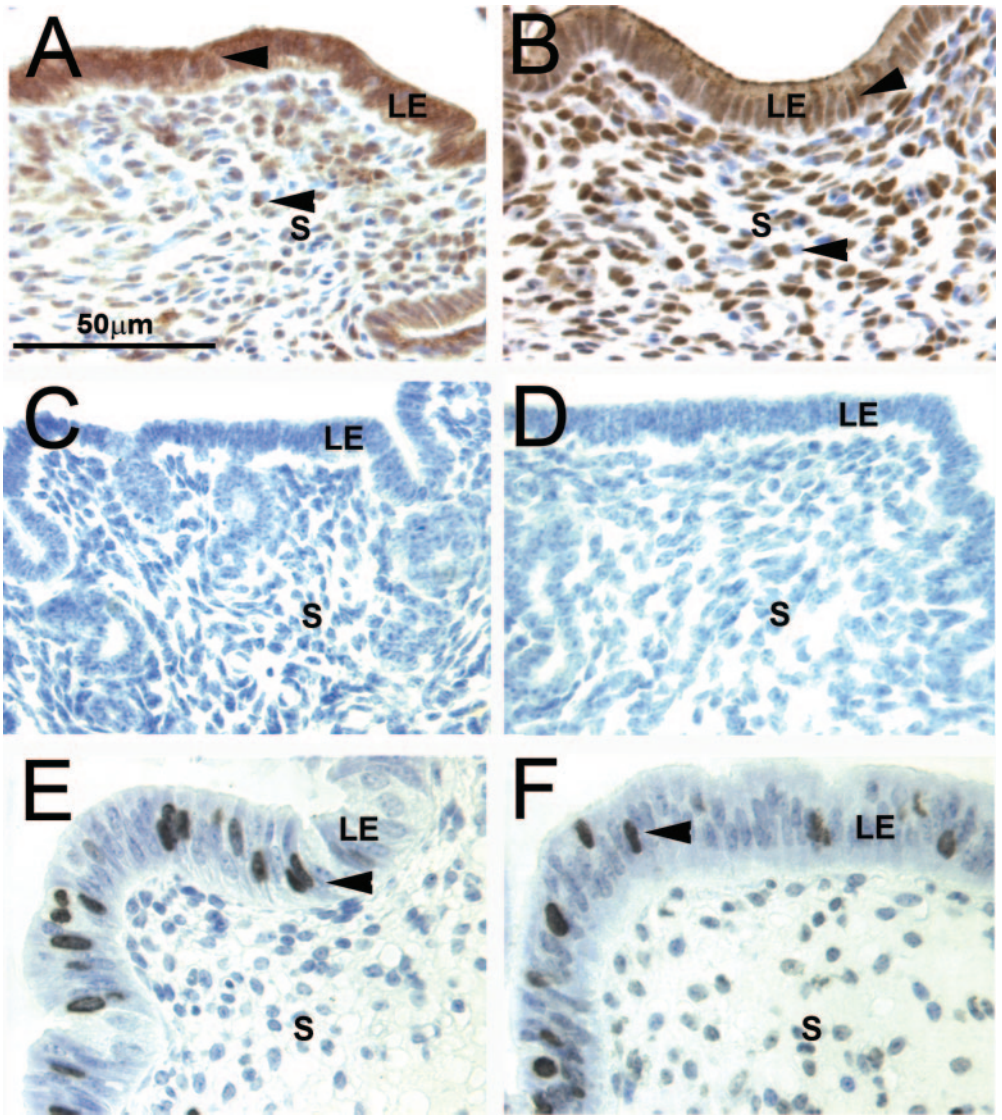


FIG. 7. Normal E-induced luminal epithelial proliferation in the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mouse uterus. (A and B) Immunohistochemical detection of ER (black arrowheads) in the luminal epithelial (LE) and stromal (S) compartments of the uteri of untreated ovariectomized WT and $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mice, respectively. (C and D) Uterine sections stained for BrdU incorporation from untreated ovariectomized WT and $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mice, respectively. (E and F) BrdU-stained sections obtained from E-treated $SRC\text{-}2^{flox/flox}$ and $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mice, respectively. Note the increase in luminal epithelial proliferation (black arrowhead) and the appearance of an edematous stroma in both panels, hallmarks of unopposed of E action. The scale bar in panel A applies to all of the panels.

$PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ bigenic mouse was generated to circumvent the complex reproductive and metabolic phenotypes of the $TIF2^{-/-}$ mouse by abrogating SRC-2 function in cell lineages that specifically express the PR.

SRC-2 is required for uterine implantation and decidualization. The coexpression of SRC-2 and PR in many cell lineages of the ovary, oviduct, uterus, and lower reproductive tract (coupled with previous $TIF2^{-/-}$ data reporting a severe uterine defect [10]) suggested that this coactivator occupies an important role in PR-mediated transcriptional programs required to maintain female fecundity. Experiments in this study revealing an infertility phenotype in the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ female strongly support this assertion. With the absence of an ovarian defect to explain why the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ female is infertile, analysis focused on whether the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ uterus is capable of undergoing the developmental changes required for embryo implantation and subsequent decidualization.

Implantation can only occur when the developmental progression of the hatched embryo to the activated blastocyst stage is synchronized with the differentiation of the uterus to the receptive state (43). Here we demonstrate that functional abrogation of SRC-2 in PR-positive uterine cells results in total failure of the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ uterus to support blastocyst implantation. The implantation phenotype not only explains why the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ female is infertile but markedly distinguishes this KO from other SRC KOs which do not exhibit implantation failure or an infertility phenotype. Apart

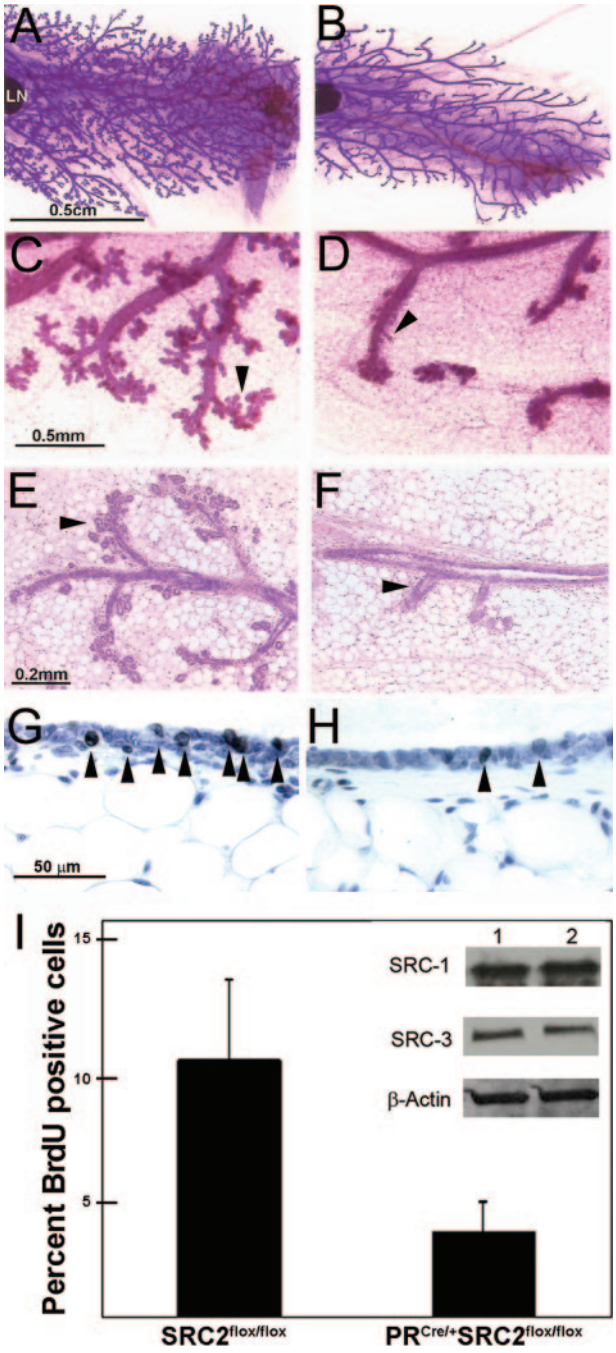


FIG. 8. Marked reduction in mammary ductal side branching and alveologenesis in the E-P-treated $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mouse. (A and B) Whole mounts of inguinal mammary glands from E-P-treated $SRC\text{-}2^{flox/flox}$ and $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mice, respectively (LN, lymph node). (C and D) Higher magnifications of regions of panels A and B, respectively. Compared to the E-P-treated $SRC\text{-}2^{flox/flox}$ mouse gland, note the significant reduction in ductal side branching and alveologenesis (black arrowhead) in the E-P-treated $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mouse gland. (E and F) Hematoxylin-and-eosin-stained sections of glands shown in panels A and B, respectively; compared to the E-P-treated $SRC\text{-}2^{flox/flox}$ mouse gland, note the marked decrease in epithelial content in the similarly treated $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mouse gland (arrowhead). In contrast to the E-P-treated $SRC\text{-}2^{flox/flox}$ mouse gland (G, arrowheads), note the significantly lower number of luminal epithelial cells scoring positive for BrdU incorporation (arrowhead) in the E-P-treated $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mouse gland (H). (I) Average percentages of mammary epithelial cells (± the standard deviations) scoring positive for BrdU staining in E-P-treated $SRC\text{-}2^{flox/flox}$ and $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mouse glands. The inset displays a Western blot assay for mammary SRC-1 and -3 in $SRC\text{-}2^{flox/flox}$ (no. 1) and $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ (no. 2) mice. Significant alterations in the levels of SRC-1 and -3 were not detected in the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mouse mammary gland (β-actin was used as a loading control). The scale bars in panels A, C, E, and G apply to panels B, D, F, and H, respectively.

from recent reports ascribing implantation defects in KO models to two "PR interacting proteins" (35, 40), our study is the first to highlight the indispensable coactivator role of SRC-2 in PR-dependent uterine responses that lead to embryo implantation. From a clinical perspective, recurrent implantation failure is now considered an important limiting factor in the establishment of pregnancy either by natural means or by assisted reproductive technologies (30). Although little is known regarding the role of SRC-2 in the human endometrium, one report has described abnormal elevations in SRC-2 levels in endometrial biopsies from infertile women with polycystic ovarian syndrome (11), suggesting a possible role for this coactivator in human uterine disorders.

The partial decidual response exhibited by the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mouse uterus suggests that uterine PR's coactivator dependency on SRC-2 quickly expands to other coactivators following implantation. The absence of a decidual response in the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ *SRC-1* KO trigenic mouse supports this conclusion and provides another example in which SRC-1 and -2 have been coselected to collaborate in transcriptional programs required for a subset of normal physiological responses (26, 32, 48). Moreover, this collaboration is reflected at the molecular level in which decidual markers Cox-2 and follistatin both require SRC-1 and -2 for full expression whereas Bmp2 expression is more dependent on SRC-2 activity.

SRC-2 is necessary for P-dependent mammary morphogenesis. Unlike the ER KO mammary phenotype (2), which consists of a developmental block at prepuberty, the PR KO gland develops normally to adulthood (24). This observation underscored the indispensable role of ER, but not PR, in the first allometric growth stage of mammary gland development, which manifests itself as ductal elongation and simple dichotomous branching at puberty. However, transplant and hormone treatment studies clearly revealed an essential role for PR in the second allometric growth phase of mammary gland development, which consists of extensive ductal side branching and alveologenesis in response to pregnancy (4, 24). Further analysis showed that these epithelial changes can only manifest in response to a PR-mediated proliferative signal (25).

Our expression studies clearly demonstrated that SRC-2 is expressed in the mammary gland and is restricted to the luminal epithelial compartment, a region directly responsive to endocrine mammogens, as well as a cellular target for neoplastic transformation (9). The detection of mammary SRC-2 in PR (and, by extension, ER)-positive cells provided support for the concept that this coactivator may be required for ER- and/or PR-mediated mammary transcriptional programs. The fact that the hormone-treated mammary gland of the adult $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mouse failed to exhibit extensive ductal

side branching and alveologenesis provides strong support for the importance of mammary SRC-2 in PR-mediated signal transduction pathways required for manifestation of the second allometric growth phase. By contrast, progression through the first allometric growth phase is unaffected in the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mouse gland, suggesting that in the mammary gland (as in the uterus) SRC-2 is required for PR (rather than ER)-mediated transcriptional programs. Similar to the PR KO phenotype, the basis of the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mammary phenotype is a marked reduction in P-induced mammary epithelial proliferation.

In both humans and rodents, immunohistochemical studies have indicated that P influences the proliferative activity of the mammary epithelium through a paracrine mechanism of action in which PR-positive mammary cells (in response to P) dispatch a paracrine signal to juxtacrine division-competent, PR-negative cells (reviewed in reference 9). Further studies have suggested that breakdown in this paracrine signaling pathway is associated with mammary tumorigenesis (6). In terms of the mechanism of action, the presence of SRC-2 in both PR-positive and -negative mammary epithelial cells suggests that this coactivator may not only directly regulate PR-mediated induction of a paracrine signal(s) but in juxtaposed PR-negative mammary epithelial cells may be required for the translation of this signal to a proliferative response. Recent reports suggest that SRC-2 may directly modulate the canonical Wnt/β-catenin pathway (20); interestingly, this signaling pathway has been described as one of the paracrine signals by which mammary PR projects its proliferative effects to nearby PR-negative cells (3).

Importantly, the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ mammary phenotype was not compensated for by SRC-3; SRC-3 is present at normal levels in the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ gland. Although SRC-3 has been shown to be involved in steroid-induced mammary morphogenesis (45), as well as tumorigenesis (1, 19, 39), our data suggest that SRC-2 and -3 are operationally distinct in the mammary epithelial cell. Irrespective of the functional interrelationships between mammary SRC-2 and other members of the SRC family, our studies reveal SRC-2 to be an important coactivator for P signaling in the mammary epithelial cell.

In sum, identification of tissue-specific coregulators that are preferentially recruited by PR in vivo constitutes one of the next important conceptual advances in our understanding of tissue selective responses to P. In this study, the $PR^{Cre/+}$ $SRC\text{-}2^{flox/flox}$ model has allowed us to conclude that SRC-2 is appropriated by PR in a subset of transcriptional programs that lead to significant proliferative and differentiative changes required for normal uterine and mammary function.

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